In vitro cytotoxicity of a composite resin and compomer

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Abstract

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Aim This work was designed to investigate the potential cytotoxicity of two of the newer dental restorative materials, Spectrum® composite resin and Dyract® AP compomer.

Methodology Cultured human endothelial cells (ECV-304) were exposed to each of the restorative materials through a 70-μm dentine barrier to simulate the *in vivo* clinical situation. Cell viability was measured by the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide) assay and lactate dehydrogenase release assay. The effects of different extents of light-curing were also examined by microscopic examination of stained human promyelocytic leukemia cells (HL-60). Caspase-3 activation was determined as a measure of apoptotic cell death.

Results Assessment of cellular viability indicated that both materials cause cell death, with Spectrum® being the more toxic. The cytotoxicity was considerably increased in the absence of the dentine barrier. Direct exposure to Spectrum® for 12 h resulted in the death of 69% of the cells after full light-curing (78% of total death was by apoptosis) and 96% after partial light-curing (73% of total death was by necrosis). Assessment of caspase activation, in the absence of the dentine barrier, showed that longer curing-times resulted in an increase in the proportion of the cells dying through apoptosis, rather than necrosis, for both materials tested.

Conclusions These results indicate the restorative materials to be potentially toxic, particularly if the degree of light-cure is inadequate.

Keywords: apoptosis, caspase, compomer, composite resin, necrosis.

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Introduction

Many new dental restorative materials have entered the market in recent years. It is claimed these products have many advantages, such as fluoride release and improved adhesion to enamel and dentine. However, in many cases, the product is released onto the market without prior independent evaluation. In this context, cytotoxicity testing is an assessment of the probability that a material will be hazardous to the body, particularly with respect to the material's potential to cause pulpal problems (Wataha *et al.* 1994).

In the past, many varieties of restorative materials have been tested and found to be hazardous. Kawahara *et al.* (1968) found that silicate and zinc phosphate cements were toxic to cultured cells, particularly early in the setting process. Meryon & Riches (1982) subsequently reported that composite resin materials were cytotoxic to fibroblasts and macrophages and Muller *et al.* (1990) found that some glass ionomer cements were toxic to primary rabbit pulpal fibroblasts.

In addition to problems arising from leakage and bacterial contamination that particularly affected silicate cements (Powis *et al.* 1988), the direct toxicity of restorative materials largely results from the elution of compounds from the material. For example, Rathbun *et al.* (1991) studied the elution of the components of composite-resin restorative materials into various organic solvents. They found that the monomer Bis-GMA (bis-glycidyldimethacrylate) was the main material eluted and they also identified a benzophenone UV stabiliser that did not participate in the photo-activated polymerization reaction. Furthermore, Geurtsen *et al.* (1998)

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showed that the elution of TEGDMA (triethylene glycol dimethacrylate) was one of the main causes for the cytotoxic reactions evoked by the light-cured glass-ionomer cements and compomers that they investigated. In fact, the National Institute of Occupational Safety and Health has classified TEGDMA as being irritating to various tissues (RTECS 1995).

It has been found that the greater the extent of the photo-initiated polymerization reaction, the fewer the monomers available to be leached (Ferracane 1994). This relationship was demonstrated by Rueggeberg & Craig (1988) for a light-cured composite consisting of the monomers Bis-GMA and TEGDMA, who showed that the degree of light-curing of this monomer mixture and the degree of conversion of carbon-carbon double bonds, to form the hardened material, was reduced by curing the composite through varying thickness of previously cured composite which reduced the light intensity.

Another significant factor to consider is whether or not the dentine provides protection for the pulp. Several features of dentine may influence the potential toxicity of a restorative material. The limited wetability of dentine limits the dissolution of the applied material, and the buffering capacity of the dentine hydroxyapaptite allows for the use of acid-base reaction materials and liquid acids (Pashley 1996). Furthermore, the presence of a smear layer may further reduce the permeability of dentine by acting as a diffusion barrier (Pashley *et al.* 1981). However, it can also be argued that the smear layer could harbour bacteria, which would then multiply beneath the applied restoration (Brannstrom 1996). Indeed Hanks *et al.* (1994), using a 'split chamber device', found that the diffusion of biological and synthetic materials through dentine was indirectly proportional to the dentine thickness. Similarly, Gerzina & Hume (1994) compared the diffusion of TEGDMA through dentine to the pulp space with its release directly into aqueous solution *in vitro* and reported that dentine provided an effective method of retarding the release of TEGDMA.

However, it is important to note that a restorative material which is hazardous *in vitro* may not necessarily be toxic *in vivo*, possibly due to biological barriers or insufficient time of contact between the offending restorations and susceptible tissues (Wataha *et al.* 1994). Therefore, in order to obtain an accurate risk assessment, the *in vitro* testing model must reflect the clinical situation as closely as possible. Two main strategies have been employed to date. The first is testing of the components of materials to cells in monolayer culture, constructing dose–response curves and then using this to estimate the cytotoxic potential of these components *in vivo*; secondly, the use of barriers between the material and the cells to mimic barriers which might exist *in vivo* (Wataha *et al.* 1994). In the present study the second strategy was used, in which a dentine barrier was placed between cultured cells and the materials to be tested *in vitro* in order to simulate possible pulpal effects in the clinical situation.

All cells have the ability to die through activation of an internally encoded suicide programme. Once activated, this programme initiates a characteristic form of cell death called apoptosis (Thompson 1995). One of the essential features of apoptosis is that, although the membrane is thrown into massive convolutions (blebbing), it remains intact. The dead cells are rapidly removed through engulfment by macrophages and any leakage of their noxious contents and consequent inflammatory response is avoided (Cotter & Martin 1995). In contrast, necrosis is a pathological form of cell death that results from an overwhelming cellular injury. It is associated with an early loss of cell membrane integrity, resulting in leakage of cytoplasmic contents and the induction of an inflammatory response. Unlike apoptosis, where there is controlled auto-digestion of the cell, necrotic cells spill their contents out over the surrounding cells, thus spreading any toxic or infectious agent and resulting in a spreading wave of necrosis (Thompson 1995).

There have been few studies of the mode of cell death induced by dental restorative materials. In view of this, morphological and enzymatic assessments of the mode of cell death were performed after light curing samples of restorative materials for different time periods.

Materials and methods

Maintenance and storage of cells

Two different mammalian cell lines were obtained from the European Collection of Animal Cell Cultures (E.C.A.C.C.). The human endothelial cell line (ECV-304), which has also been used in previous apoptosis studies (Escargueil-Blanc *et al.* 1998), was chosen to simulate the *in vivo* situation. The human promyelocytic leukaemia cell line (HL-60) was the model used for apoptosis studies (Jarvis *et al.* 1994).

The ECV-304 adherent cells were grown in medium 199 supplemented with foetal calf serum (FCS, 10% $^{v}/_{v}$), L-glutamine (2 m mol L^{-1}), penicillin (100 U mL^{-1}) and streptomycin (100 μg mL^{-1}). The nonadherent HL-60 cells were grown in RPMI medium that had been supplemented with FCS (20% $^{v}/_{v}$), L-glutamine (2 m mol L^{-1}), penicillin (100 U mL^{-1}) streptomycin (100 μg mL^{-1}), and amphotericin B solution (2%). The stock cultures

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were maintained at 37°C in a humidified atmosphere (Forma CO_2-water-jacketed incubator, Forma Scientific, OH, USA) with 5% CO_2 in 95% air, in 75 cm^2 tissue culture flasks (Grenier® Laboratories, Frickenhausen, Germany). The ECV-304 cells were passaged twice weekly, upon reaching confluence and re-seeded at a cell density of 1.0×10^5 cells mL^{-1}. The HL-60 cells were passaged three times weekly and re-seeded at a cell density of 1.5×10^5 cells mL^{-1}.

Moulds and dentine preparation

Human maxillary canine teeth (10), which had been extracted for orthodontic reasons, were obtained with patient consent (from the Dublin Dental Hospital) and stored in physiological saline until required. Both the crown and root portions of the teeth were embedded whole in clear methyl methacrylate resin under vacuum. The crown portion was later sectioned into 70-micron sections, using a hard tissue sectioner (Leica AG, Bensheim, Germany) under water spray. The dentine of the root portion was not used experimentally.

To ensure reproducible dimensions of sample materials employed – Spectrum® composite resin (Dentsply, Surrey, UK) and Dyract® AP compomer (Dentsply) – a custom mould was created so that material discs of 3 mm diameter and 2 mm thickness were produced. The mould was constructed in additional vinyl polysiloxane impression material (3M Express®, 3M Dental Products, MN, USA).

Each dentine section was cut into a diameter similar to that of a well from a 96 well plate and then etched for 30 s using 37% phosphoric acid gel, washed for 30 s in water, dried and then placed on a sterile glass block. Primer (Prime & Bond®, OSB Bond®, Dentsply) was dispensed into a plastic watch-glass and applied with a brush to the dentine section to form a thin layer and then light-cured for 10 s using a Visilux 2 visible-light curing unit (3M Dental Products).

A second layer of primer was applied and light-cured, as before, for 10 s. The mould was then placed centrally on the dentine section and the sample material (Spectrum®, Dyract® AP) was dispensed into the mould using a preloaded compule tip gun. The material was then fully cured for 40 s.

The mould was removed and a layer of soft red wax was placed around three sides of the restorative material attached to the centre of the dentine section, the fourth remained free of wax to allow for the passage of air and CO_2 to the underlying cells. The samples were inserted into the plate well so that the dentine, in contact with the culture medium, formed a physical barrier between the restorative material and the cells. This was achieved via the wax seal, which was mouldable at room temperature, allowing the sample to be lowered into position.

The above system was used for studies of cell viability by the MTT (3,-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) reduction and the LDH (lactate dehydrogenase) release procedures with ECV-304 cells, as described below.

For studies with HL-60 cells no dentine sections were used. A custom mould was created, as described above, so that material samples of 3 mm diameter and 1 mm thickness were produced, and the mould was placed directly onto the sterile glass block. The restorative material (Spectrum® or Dyract® AP) was dispensed, using the preloaded compule gun, into the well of the mould and light-cured for 1 s (partially cured), 4 s (until the restoration was solid to probing) or 40 s (fully cured). The mould was then removed and the materials were stored in sterile containers in the dark until required.

Cell viability assays

For both the LDH and MTT assays, ECV-304 cells were seeded, at a density of 1.0×10^5 cells mL^{-1}, into three 96-well microplates and incubated at 37°C in 5% CO_2, 95% air and left to adhere overnight. A fully cured sample of Dyract® AP or Spectrum® was inserted into each well so that the dentine disc with the attached material was in direct contact with the culture medium, leaving a space of approximately 5 mm between the sample and cell population. In addition, the samples were held in place by a wax seal. Controls of wax and dentine alone (70 µm thick, surrounded by wax) were added in triplicate to each plate. As before, controls were in contact with the culture medium. Each plate was further incubated at 37°C in 5% CO_2, 95% air over a period of 1–3 days.

Cell viability was determined using the MTT assay (Mosmann 1983). Upon incubation with viable cells, the tetrazolium ring of MTT (pale yellow) is cleaved by cellular dehydrogenases and at sites in the mitochondrial electron-transport system (Vistica *et al.* 1991) to yield a purple formazan product. MTT solution (0.5 mg mL^{-1} per well) was added to each plate and they were incubated for 2.5 h at 37°C in the dark. The formazan crystals were solubilized with DMSO (200 µL, dimethylsulfoxide) and the absorbance determined at $\lambda = 595$ nm using an ELISA plate reader ($Thermo_{max}$ Microplate Reader, Molecular Devices, CA, USA).

Cell lysis was determined using an LDH assay kit under conditions described by the manufacturer (Promega Corporation, Madison, WI, USA). The essence of this

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assay is that LDH is a cytosolic enzyme that is released upon cell lysis. The released LDH is measured in a coupled assay that results in the conversion of a tetrazolium salt into a red formazan product. The amount of colour formed, expressed as a percentage of the total LDH activity, is proportional to the number of lysed cells. Total LDH was determined after lysis of untreated cells using the detergent, Triton® X-100 (9% $^{v}/_{v}$, Korzeniewski & Callewaert 1983). Following sample incubation, 50 µL aliquots were transferred from all wells to a fresh 96-well plate. To each well 50 µL of the reconstituted substrate mix was added and incubated for 30 min at room temperature. Stop solution (50 µL) was added to each well and the absorbance was measured within 1 h at $\lambda = 490$ nm using an ELISA plate reader. Both the LDH and MTT assays were carried out in triplicate and repeated in three separate experiments.

Morphological staining of cells

HL-60 cells were seeded, at a density of 5.0×10^5 cells mL, into a sterile 96-well microplate and incubated at 37°C in a humidified atmosphere (Forma CO_2-water-jacketed incubator) with 5% CO_2 in 95% air overnight. The following day 20 fully cured or partially cured samples (one sample per well) of Dyract® AP or Spectrum® were added to the plates, whilst the control remained untreated and the cells were further incubated over a period of 2–12 h.

Every 2 h one of each sample type was removed and the cells (150 µL; 5.0×10^5 cells mL^{-1}) were loaded into the prepared cytospin cup and slide set-up and cytocentrifuged. The slides (Shandon Corporation, PA, USA) were then air-dried at room temperature before being differentially stained using the RAPI-DIFF II stain pack (Diachem International Ltd, Lancashire, UK). The slides were allowed to air-dry and then viewed by light microscopy under ×40 magnification. Apoptotic cells were characterized by cell shrinkage, membrane blebbing, nuclear condensation and fragmentation, whilst necrotic cells were identified by cell swelling and loss of cell membrane. The percentage of apoptotic and necrotic cells was determined by taking counts of 100 cells in three separate fields of view and correlating this to the total number of cells present. This procedure was carried out in triplicate and repeated three times for the fully and partially cured materials.

Determination of caspase-3 activity

Caspases, a family of cysteine proteases, are thought to play a vital role in the disassembly of the cell during apoptosis. These enzymes ensure the ultimate demise of the cell by preventing DNA repair, condensing the cytoplasm and inhibiting DNA synthesis and splicing (Villa *et al.* 1997). Caspase-3 activity was determined using the fluorometric peptide substrate Asp-Glu-Val-Asp-Aminomethyl Coumarin (DEVD-AMC, Alexis Corporation, Nottingham, UK) which contains the specific aspartic acid cleavage site for this peptidase. The caspase-3 activity was quantified as the amount of the fluorescent leaving group (AMC) released per min per mg of protein, according to the method of Kaushal *et al.* (1997).

The HL-60 cells were seeded at a density of 5.0×10^5 cells mL^{-1} into 96-well microplates and incubated at 37°C in 5% CO_2, 95% air for 24 h. Thirty samples each of Dyract® AP and Spectrum® that had been cured for either one, four or 40 s were added to one of the 96-well plates and incubated in a humidified atmosphere at 37°C in 5% CO_2, 95% air for a further 10 h. The control consisted of untreated cells. For the etoposide treatment, 4 h prior to harvesting the cells, following completion of sample incubation, etoposide (50 µ mol L^{-1}) was added to 5 mL of HL-60 cells (5.0×10^5cells mL^{-1}). This was used as a positive control, because etoposide is a DNA-damaging agent that induces the structural changes associated with apoptosis (Ritke *et al.* 1994).

Following sample incubation, the cells were pelleted by centrifugation, washed in PBS, and recentrifuged. The pellets were resuspended in 20 m mol^{-1} HEPES (N-[2-hydroxyethyl] piperazine-N′-[2-ethanesulfonic acid), pH 7.5, containing 10% sucrose, 0.1% CHAPS (3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate), 0.1% NP40 (Nonidet P40), 1 m mol L^{-1} EDTA (ethylenediaminetetraacetic acid), 1 m mol L^{-1} PMSF (phenylmethylsulfonyl fluoride), 2 m mol L^{-1} DTT (DL-dithiothreitol), 1 µg mL^{-1} leupeptin and 1 µg mL^{-1} pepstatin.

The supernatants obtained after centrifugation were used to determine the enzyme activity. A 50-µg sample of protein from each set of samples was incubated with 100 m mol L^{-1} HEPES, pH 7.5 (containing 10% sucrose, 0.1% CHAPS, 10 m mol L^{-1} DTT) and 20 µm DEVD-AMC substrate in a total reaction volume of 3 mL. After incubation for 60 min at 25°C, the fluorescence was determined using a spectrophotofluorometer (Perkin-Elmer Corporation, Ueberlingen, Germany) at an excitation wavelength of 380 nm and an emission wavelength of 460 nm. The amount of fluorescence from the cells was correlated to the amount of liberated AMC using an AMC calibration curve. Protein concentrations of the samples were determined by the Bradford (1976) method.

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Statistics

Statistical significance was determined by Student's *t*-test with a *P*-value of <0.05 being considered significant.

Results

In pilot studies, examination under the light microscope (×40) indicated that when dentine sections were placed directly on the surface of the cell-culture medium they sank to the base of the well within about 5 min, disturbing some of the adherent cells. Thus it was necessary to melt a layer of soft wax around three sides of the material attached to the dentine, to ensure that it was in contact with the culture medium but did not sink down to the base of the well.

MTT assay

The MTT assay was used to determine the effect of each restorative material on cell viability over a three-day time period. The restorative materials had little effect on day 1 (Fig. 1). However, after 2 days of incubation, Spectrum® resulted in a significant reduction in cell number relative to the dentine control ($P < 0.01$), whereas Dyract® AP had no significant effect. By the third day both materials had caused significant reductions in viable cells, with Spectrum® ($P < 0.01$) being a more potent inhibitor than Dyract® AP ($P < 0.05$).

LDH assay

The relationship between material cytotoxicity and cell viability was further investigated using the release of LDH from cultured cells (Fig. 2). This is a cytosolic enzyme that is only released from lysed cells. There was no release of LDH from the treated cells on the first day. Although some LDH release could be detected after 2 days' incubation, this was not statistically different from the control samples. However, after 3 days of incubation Spectrum® resulted in substantial cell lysis (47.4% of total LDH),

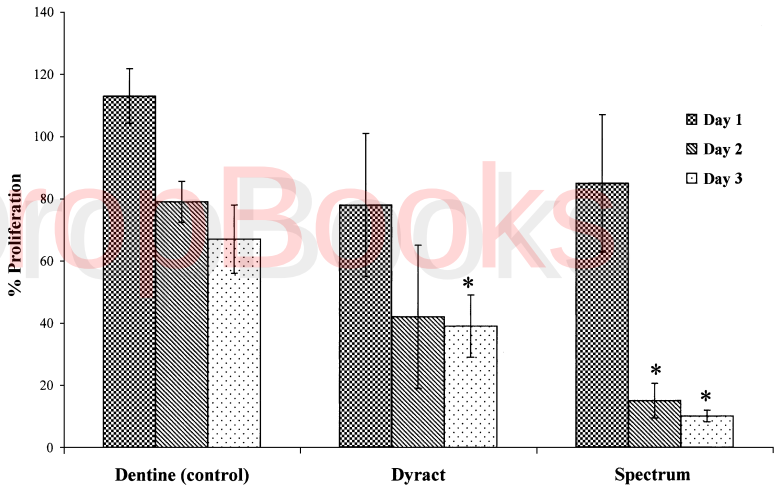

Figure 1 Cell viability of ECV-304 cells following exposure to restorative materials. Viability was assessed using the MTT assay. *, denotes significant difference from the control (dentine), $P < 0.05$ (Dyract), $P < 0.01$ (Spectrum).

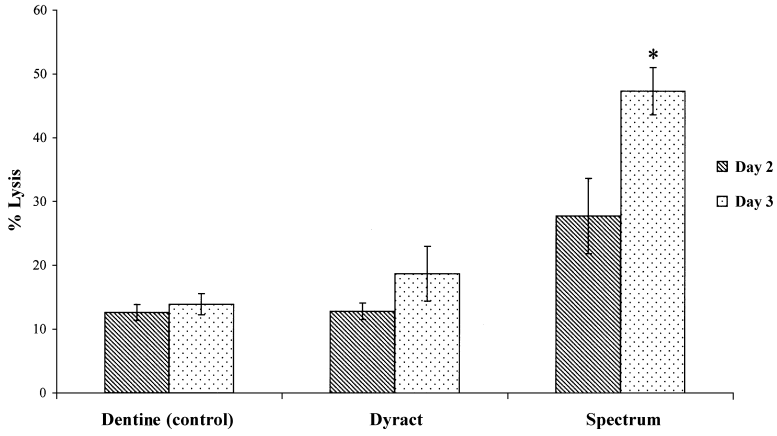

Figure 2 Cell lysis ECV-304 cells following exposure to restorative materials measured by LDH release. *, denotes significant difference from the control (dentine) $P < 0.01$ (Spectrum).

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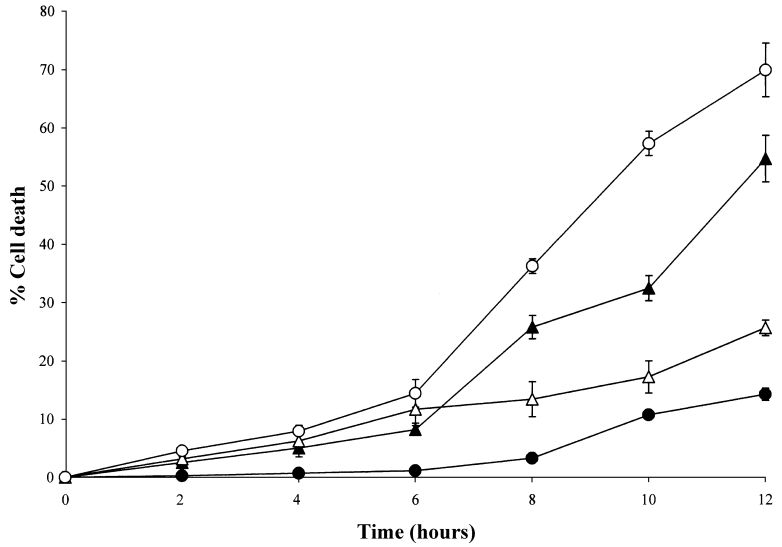

Figure 3 Time-course of the death of HL-60 cells exposed to Spectrum® composite resin. Cells were incubated with fully cured (▲,●) and partially cured (△,○) samples. Apoptosis (△,▲) and necrosis (○,●) were determined microscopically (×40).

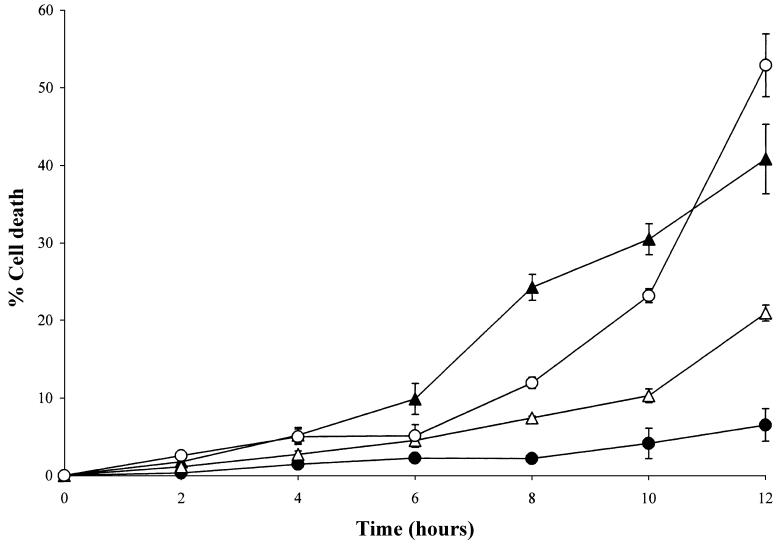

Figure 4 Time-course of the death of HL-60 cells exposed to Dyract AP® compomer. Cells were incubated with fully cured (▲,●) and partially cured (△,○) samples. Apoptosis (△,▲) and necrosis (○,●) were determined microscopically (×40).

whereas Dyract® AP (18.7% of total LDH) was less potent.

Morphological staining of cells

Fully (40 s) and partially (4 s) cured samples of Dyract® AP and Spectrum® were incubated with HL-60 cells over a period of 2–12 h. Unlike the previous studies, where the materials were separated from the cells by dentine, each material sample was in direct contact with the cells in this case. This resulted in an increase in cell death over the 12 h (Figs 3, 4) for both the partially and fully cured sample materials. When fully cured material was used, the most common form of cell death was by apoptosis (Spectrum® 55%, Dyract® AP 40%) with little necrosis being evident (Spectrum® 14%, Dyract® AP 6%) at the end of the 12 h incubation. However, with partially cured material, there was a marked reduction in the level of apoptotic cell death (Spectrum® 26%, Dyract® AP 20%) with necrosis becoming more prevalent (Spectrum® 70%, Dyract® AP 53%).

Caspase-3 fluorogenic assay

The process of apoptotic cell death is often associated with activation of the peptidase, caspase-3. No significant activity was observed in the control (untreated) cells. The fully cured (40 s) samples resulted in the greatest

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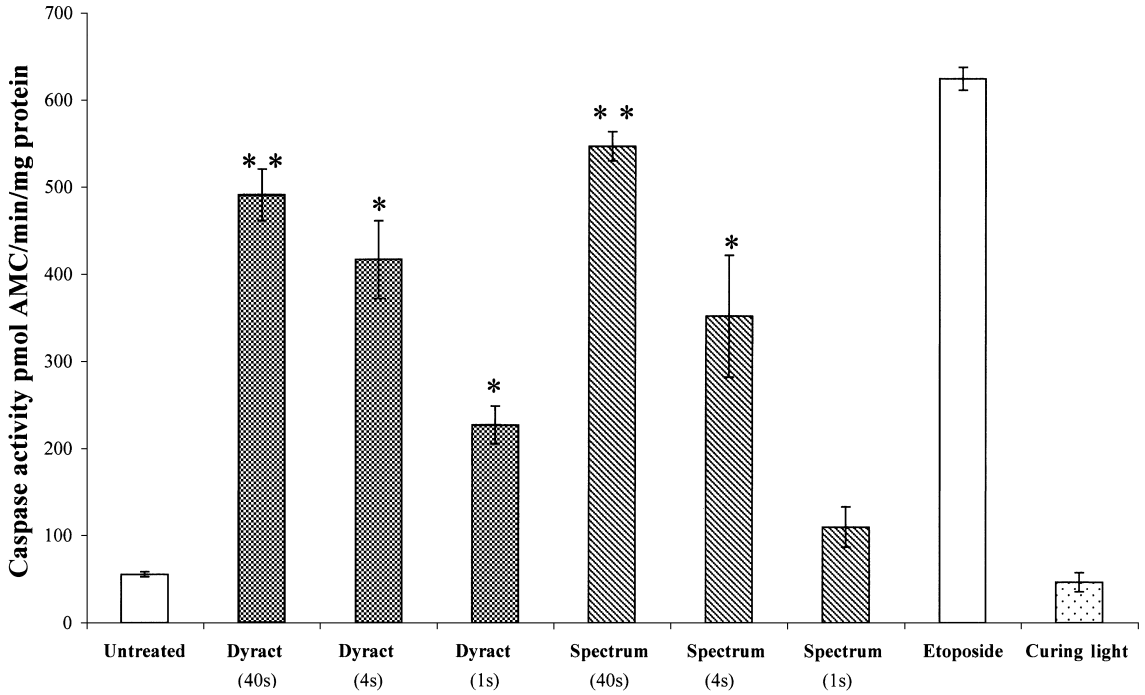

Figure 5 Caspase activity in HL-60 cells after exposure to restorative materials. *, Denotes significant difference from the control, $^{**}P < 0.0001$, $^{*}P < 0.05$ compared to the untreated cells and curing light alone.

caspase-3 activation, relative to the positive, etoposide-treated controls (Fig. 5), with Spectrum® (547 pmol AMC min^{-1} mg^{-1} protein) having a greater effect than Dyract® AP (490 pmol AMC min^{-1} mg^{-1} protein). The extent of caspase-3 activation increased with the time of light-curing, with partially cured Dyract® AP (caspase-3 activities of 227 and 401 pmol AMC min^{-1} mg^{-1} protein after 1 and 4 s light-curing, respectively) being more potent than Spectrum® (110 and 352 pmol AMC min^{-1} mg^{-1} protein, respectively).

Discussion

A dentine barrier, simulating the clinical situation, was used to determine whether the two dental restorative materials were cytotoxic *in vitro*. The dentine disc acted as a physical barrier between the restorative material and the underlying culture medium and cells. However, a dentine thickness of only 70 microns was chosen, because a barrier of increased thickness may mask the material's potential cytotoxicity. Earlier work by Tyas (1977) using dentine chips showed the toxicity of zinc phosphate cement to be substantially reduced by such a barrier. Although it is not possible to make a direct comparison between two different cell lines, this would appear consistent with the more rapid appearance of toxicity in the HL-60 cells incubated in the absence of the dentine barrier, where both materials resulted in substantial cell death after 12 h, as compared to the adherent ECV-304 cell-line in the presence of the barrier, where substantial toxicity was not observed until after 2 or 3 days of incubation. To mimic the clinical situation more closely, primer was placed on the dentine prior to placement of the restorative material. This layer forms a thin barrier between the restorative and the pulp. However, the effectiveness of this barrier, in terms of limiting ingress from the restorative material, is not known.

Whereas the MTT assay is a good indicator of cell viability, the LDH assay is an indicator of cell lysis. The results of these two assays revealed that, in the presence of the 70 μm dentine barrier, Dyract® AP had no effect upon cell lysis until the third day of incubation, whereas the toxic effects of Spectrum® were evident after only 2 days' incubation. Thus the cytotoxicity exerted through dentine slices by the Dyract® AP restorative material was less than that of Spectrum®, which was extremely cytotoxic and more likely to evoke an adverse pulpal reaction.

During cavity preparation, prior to placement of the restorative material, the mechanical action of the bur may cause microclefts in the dentine. In such cases, the

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restorative material would be in direct contact with the pulpal tissue, thus magnifying the potential to evoke an adverse pulpal response (Swift *et al*. 1995). In the present work a suspensory cell-line (HL-60) was used, in the absence of the dentine barrier, for studies on such direct toxicity and on the mechanisms underlying the toxicity of these restorative materials.

Microscopic examination of the stained cells revealed both restorative materials cause cell death, with Spectrum® being more toxic than Dyract® AP. When fully cured materials (40 s light-curing) were used, the predominant form of cell death was apoptosis. In contrast, the main form of cell death following exposure to partially cured materials (4 s light-curing) was necrosis.

Fully cured samples of Spectrum® and Dyract® AP caused activation of caspase-3. Thus, in agreement with the morphological staining data, the fully cured samples induce apoptotic cell death. However, exposure to the partially cured (1 and 4 s) materials resulted in less caspase activation than the fully cured materials. This contrasts with the morphological staining results that showed the partially cured samples to be more toxic than the fully cured material. This would indicate that whilst the partially cured samples cause some apoptosis, the materials provide such an overwhelming insult that the majority of the cells die rapidly by necrosis, and the less cured the material is, the greater the toxicity. Apoptosis and necrosis have been shown to occur together in some other systems. For example, in myocardial infarction cell death is associated with rapid necrosis immediately around the central ischaemic zone, whereas outside the central zone cells die more slowly by apoptosis (Cohen 1993).

Even in fully set restorative materials substantial amounts of short-chain polymers remain unbound, with the result that there is possible elution of leachable toxic components toward the pulp (Ferracane 1994). This may be compounded by inadequate light-curing. In some instances the light-curing unit may emit light that is not at the optimum wavelength (490 nm) for complete setting of the material (Miyazaki *et al*. 1998). As a result, the restoration might appear solid to probing but would in fact, be incompletely set and capable of initiating adverse pulpal reactions. Poor awareness of the need for regular maintenance of the light intensity of light-curing units amongst dentists has been reported (Martin 1998). In the clinical situation necrotic cell death would result in an inflammatory response and possibly secondary tissue damage to the patient. The present findings indicate that the degree of light-cure is an important factor in the toxic potential of a material, where an inverse relationship exists between the degree of light-curing and restoration cytotoxicity.

Conclusions

In the presence of a dentine barrier the restoratives Spectrum® and Dyract® AP are both cytotoxic with the former having the greater toxicity. The toxicity toward cultured cells was greater in the absence of the dentine barrier. Inadequate light-curing resulted in an enhanced toxicity of these materials. Whereas apoptosis was the predominant form of cell death induced by the fully cured materials, necrosis was the main form of cell death resulting from exposure to the partially cured materials. Thus, both the composite resin and the compomer restorative materials used in this study were cytotoxic, and inadequate light-curing and direct access to the pulp would greatly increase the possibility for adverse pulpal reactions.

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